

## Reference

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**Xylanase and  $\beta$ -xylosidase production: alternatives for the autohydrolysis liquor application**

Michelin, Michele (1,2); Polizeli, Maria de Lourdes T. M. (2); Silva, Daniel P. (1,3); Ruzene, Denise S. (1,3); Vicente, António A. (1); Jorge, João A. (2); Terenzi, Héctor F. (2); Teixeira, José A. (1)

1: IBB - Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal;

2: Department of Biology, Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, University of São Paulo, 14040-901 Ribeirão Preto-SP, Brazil;

3: ITP - Instituto de Tecnologia e Pesquisa, Tiradentes University, Campus Farolândia, 49032-490 Aracaju-SE, Brazil

E-mail: silvadvp@deb.uminho.pt

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**Abstract**

Research into microbial xylanases production is a required development as these enzymes have several important industrial applications (e.g. bioconversion of lignocellulosic waste into their constituent sugars). Treatments of lignocellulosic materials in autohydrolysis processes, under optimized conditions, lead to the solubilization of hemicelluloses (liquid phase, liquor), allowing for the use of fractions remaining (cellulose/lignin) in other bioprocess, reducing industrial costs of the whole raw material. For this reason, wheat straw autohydrolysis liquor may be considered as a fermentation medium adjunct for the production of xylanase and  $\beta$ -xylosidase by strain *Aspergillus ochraceus* in submerged cultivation containing wheat bran as substrate. For the preparation of the liquor, wheat straw was milled, sieved (1.0 mm screen), dried, homogenized and stored. Extraction was done by adding water to the wheat straw sample in a closed and pressurized vessel (solid/liquid ratio of 1:10w/w), and heating the system to 200°C for 30 min. The obtained liquor (hemicelluloses rich fraction) was separated from the solids by filtration. The hemicelluloses were then precipitated with three volumes of 95% ethanol (20°C/24 h) and dried for yield determination (4.9%), or used directly as liquid substrate. Adams medium was used for cultivation with different carbon sources: 1%w/v birchwood xylan; 0.5%w/v wheat bran; 1%w/v wheat bran; and combination of 1%w/v wheat bran and 10%v/v liquor. The cultivation conditions were 30°C/100 rpm, for 7 days. Xylanase was assayed by DNS, while  $\beta$ -xylosidase by released *p*-nitrophenolate using 1% birchwood xylan as substrate in citrate-phosphate buffer, pH 6.0 for xylanase determination and 0.25% pnp- $\beta$ -D-xyloside in citrate-phosphate buffer pH 4.5 for  $\beta$ -xylosidase. One unit of enzymatic activity was defined as the amount that liberated 1 mmol of product per minute on assay conditions. The results showed that the use of the liquor as an alternative substrate to xylanase production allowed for the obtention of values similar to those using 1% wheat bran until 96 h, decreasing after this time. However, in the case of  $\beta$ -xylosidase production, the liquor had a positive effect as, after the first 24 h of cultivation higher activities values were obtained, allowing to conclude on the interest of its use as an alternative substrate for improved  $\beta$ -xylosidase production.

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